

**WHAT IS CLAIMED IS:**

- 1                   1.    A method of sialylating a saccharide group on a recombinant  
2 glycoprotein, the method comprising contacting a saccharide group which comprises a  
3 galactose or N-acetylgalactosamine acceptor moiety on a recombinant glycoprotein with a  
4 sialic acid donor moiety and a recombinant sialyltransferase in a reaction mixture which  
5 provides reactants required for sialyltransferase activity for a sufficient time and under  
6 appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said  
7 saccharide group.
- 1                   2.    The method of claim 1, wherein the sialic acid donor moiety is CMP-  
2 sialic acid.
- 1                   3.    The method of claim 2, wherein the CMP-sialic acid is enzymatically  
2 generated *in situ*.
- 1                   4.    The method of claim 1, wherein the sialyltransferase is a recombinant  
2 eukaryotic sialyltransferase which substantially lacks a membrane-spanning domain.
- 1                   5.    The method of claim 1, wherein the sialyltransferase includes a sialyl  
2 motif which has an amino acid sequence that is at least about 40% identical to a sialyl motif  
3 from a sialyltransferase selected from the group consisting of ST3Gal I, ST6Gal I, and  
4 ST3Gal III.
- 1                   6.    The method of claim 1, wherein the sialyltransferase is a recombinant  
2 ST3Gal III.
- 1                   7.    The method of claim 6, wherein the sialyltransferase is a recombinant  
2 rat ST3Gal III.
- 1                   8.    The method of claim 1, wherein the sialyltransferase is a recombinant  
2 ST3Gal IV.

1 9. The method of claim 1, wherein the sialyltransferase is a recombinant  
2 ST6Gal I.

1 10. The method of claim 1, wherein the sialyltransferase is a recombinant  
2 ST3Gal I.

1 11. The method of claim 10, wherein the reaction mixture comprises a  
2 second recombinant sialyltransferase, which second recombinant sialyltransferase is an  
3 ST3Gal III.

1 ~~Sub B5~~ 12. The method of claim 1, wherein the sialyltransferase is a recombinant  
2 bacterial sialyltransferase.

1 13. The method of claim 12, wherein the bacterial sialyltransferase has an  
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a *Neisseria*  
3 *meningitidis* 2,3-sialyltransferase.

1 14. The method of claim 13, wherein the bacterial sialyltransferase is a  
2 *Neisseria meningitidis* 2,3-sialyltransferase.

1 15. The method of claim 12, wherein the bacterial sialyltransferase has an  
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3 *Photobacterium damsela* 2,6-sialyltransferase.

1 16. The method of claim 15, wherein the bacterial sialyltransferase is a  
2 *Photobacterium damsela* 2,6-sialyltransferase.

1 17. The method of claim 12, wherein the bacterial sialyltransferase has an  
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3 *Haemophilus* 2,3-sialyltransferase.

1 18. The method of claim 17, wherein the sialyltransferase is a *Haemophilus*  
2 2,3-sialyltransferase.

1 19. The method of claim 12, wherein the bacterial sialyltransferase has an  
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3 *Campylobacter jejuni* 2,3-sialyltransferase.

1 20. The method of claim 19, wherein the sialyltransferase is a  
2 *Campylobacter jejuni* 2,3-sialyltransferase.

1 21. The method of claim 1, wherein the sialyltransferase is produced by  
2 recombinant expression of a sialyltransferase in a host cell selected from the group  
3 consisting of an insect cell, a mammalian cell, and a fungal cell.

1 22. The method of claim 21, wherein the host cell is an *Aspergillus niger*  
2 cell.

1 23. A method of sialylating a saccharide group on a recombinant  
2 glycoprotein, the method comprising contacting a saccharide group which comprises a  
3 galactose or an N-acetylgalactosamine acceptor moiety on a recombinant glycoprotein with a  
4 sialic acid donor moiety and a bacterial sialyltransferase in a reaction mixture which  
5 provides reactants required for sialyltransferase activity for a sufficient time and under  
6 appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said  
7 saccharide group.

1 24. The method of claim 23, wherein the bacterial sialyltransferase has an  
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3 *Photobacterium damsela* 2,6-sialyltransferase.

1 25. The method of claim 24, wherein the bacterial sialyltransferase is a  
2 *Photobacterium damsela* 2,6-sialyltransferase.

1 26. The method of claim 23, wherein the bacterial sialyltransferase has an  
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a *Neisseria*  
3 *meningitidis* 2,3-sialyltransferase.

1 27. The method of claim 26, wherein the sialyltransferase is a *Neisseria*  
2 *meningitidis* 2,3-sialyltransferase.

1 28. The method of claim 23, wherein the bacterial sialyltransferase has an  
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3 *Campylobacter jejuni* 2,3-sialyltransferase.

1 29. The method of claim 28, wherein the sialyltransferase is a  
2 *Campylobacter jejuni* 2,3-sialyltransferase.

1 30. The method of claim 23, wherein the bacterial sialyltransferase has an  
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3 *Haemophilus* 2,3-sialyltransferase.

1 31. The method of claim 30, wherein the sialyltransferase is a *Haemophilus*  
2 2,3-sialyltransferase.

1 32. A method for *in vitro* sialylation of saccharide groups present on a  
2 glycoprotein, said method comprising contacting said saccharide groups with a  
3 sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase  
4 activity for a sufficient time and under appropriate conditions to transfer sialic acid from said  
5 sialic acid donor moiety to said saccharide group, wherein said sialyltransferase is present at  
6 a concentration about 50 mU per mg of glycoprotein or less.

1 33. The method of claim 32, wherein the sialyltransferase is present at a  
2 concentration of between about 5-25 mU per mg of glycoprotein.

1           34. The method of claim 32, wherein the sialyltransferase is present at a  
2 concentration of between about 10-50 mU/ml of reaction mixture and the glycoprotein is  
3 present in the reaction mixture at a concentration of at least about 2 mg/ml.

1           35. The method of claim 32, wherein the method yields a glycoprotein  
2 having sialylation of at least about 80% of terminal galactose residues present on the  
3 saccharide groups.

1           36. The method of claim 32, wherein the sialyltransferase is a recombinant  
2 sialyltransferase.

1           37. The method of claim 36, wherein the sialyltransferase substantially  
2 lacks a membrane-spanning domain.

1           38. The method of claim 32, wherein the sialyltransferase includes a sialyl  
2 motif which has an amino acid sequence that is at least about 40% identical to a sialyl motif  
3 from a sialyltransferase selected from the group consisting of ST3Gal I, ST6Gal I, and  
4 ST3Gal III.

1           39. The method of claim 32, wherein the sialyltransferase is an ST3Gal III.

1           40. The method of claim 39, wherein the ST3Gal III is a rat ST3Gal III.

1           41. The method of claim 32, wherein the sialyltransferase is an ST3Gal IV.

1           42. The method of claim 32, wherein the sialyltransferase is an ST3Gal I.

1           43. The method of claim 42, wherein the reaction mixture comprises a  
2 second recombinant sialyltransferase, which second recombinant sialyltransferase is an  
3 ST3Gal III.

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44. The method of claim 32, wherein the sialyltransferase is a bacterial sialyltransferase.

1 45. The method of claim 44, wherein the bacterial sialyltransferase is a  
2 recombinant sialyltransferase.

1 46. The method of claim 44, wherein the bacterial sialyltransferase has an  
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a *Neisseria*  
3 *meningitidis* 2,3-sialyltransferase.

1 47. The method of claim 46, wherein the bacterial sialyltransferase is a  
2 *Neisseria meningitidis* 2,3-sialyltransferase.

1 48. The method of claim 44, wherein the bacterial sialyltransferase has an  
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3 *Photobacterium damsela* 2,6-sialyltransferase.

1 49. The method of claim 48, wherein the bacterial sialyltransferase is a  
2 *Photobacterium damsela* 2,6-sialyltransferase.

1 50. The method of claim 44, wherein the bacterial sialyltransferase has an  
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3 *Campylobacter jejuni* 2,3-sialyltransferase.

1 51. The method of claim 50, wherein the sialyltransferase is a  
2 *Campylobacter jejuni* 2,3-sialyltransferase.

1 52. The method of claim 44, wherein the bacterial sialyltransferase has an  
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3 *Haemophilus* 2,3-sialyltransferase.

1 53. The method of claim 52, wherein the sialyltransferase is a *Haemophilus*  
2 2,3-sialyltransferase.

1 54. The method of claim 32, wherein the sialic acid donor moiety is CMP-  
2 sialic acid.

1 55. The method of claim 54, wherein the CMP-sialic acid is enzymatically  
2 generated *in situ*.

1 56. The method of claim 32, wherein the sialic acid is selected from the  
2 group consisting of NeuAc and NeuGc.

1 ~~sub B8~~ 57. A method for *in vitro* sialylation of saccharide groups present on a  
2 glycoprotein, the method comprising contacting the saccharide groups with an ST3Gal III  
3 sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase  
4 activity for a sufficient time and under conditions to transfer sialic acid from said sialic acid  
5 donor moiety to said saccharide group, wherein said ST3Gal III sialyltransferase is present at  
6 a concentration of less than about 50 mU per mg of glycoprotein.

1 58. The method of claim 57, wherein the method further comprises  
2 contacting the saccharide groups with an ST6GalII sialyltransferase.

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